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Immunology

Lymphocyte function-associated antigen 1 (LFA-1): A surface antigen distinct from Lyt-2,3 that participates in T lymphocyte-mediated killing

(monoclonal antibodies/Lyt 1, CLA, T200, Ly 5, Ly 6, Thy-1, H-2, and Lgp100 antigens/M7/14 hybridoma/ xenogeneic cytotoxic T lymphocytes)

DENISE DAVIGNON*, ERIC MARTZ†, TIM REYNOLDS, KONRAD KÜRZINGER*, AND TIMOTHY A. SPRINGER*‡

Department of Pathology, Harvard Medical School, Boston, Massachusetts 02115

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ABSTRACT **Monoclonal antibodies (MAb) have been used to probe the relationship of cytolytic T lymphocyte (CTL) surface molecules to CTL function. Rat MAb to mouse CTL were generated. Twelve MAb so obtained gave preferential binding to T cells as compared to B cells, and three of these recognized previously undescribed surface polypeptides. These Mab and more broadly reactive and previously obtained MAb were tested for their ability to block CTL-mediated killing in the absence of complement. To ensure that any observed blocking was due to binding of MAb to the effector cell rather than the target cell, a xenogeneic mouse CTL anti-rat BN lymphoma target cell system was utilized (MAb and target cells both of rat origin). Of 24 MAb tested here, 21 had little or no effect on CTL function, including those to H-2, Thy-1, Lyt-1, Ly 5, Ly 6, Lgp 100, and at least six other defined antigens. We confirmed inhibition of killing with two MAb to Lyt-2,3. Another MAb, M7/14, gave profound and consistent blockade of CTL function. It was confirmed that M7/14 MAb blocks killing by binding to the mouse CTL and does not bind to the rat lymphoma target cells used for the CTL assay. The findings suggest that the antigen defined by M7/14, termed a *lymphocyte function-associated antigen*, LFA-1, participates in or is closely associated with the mechanism of CTL-mediated killing. LFA-1 contains two polypeptide chains of 180,000 and 95,000 M_r and is distinct from other described lymphocyte glycoproteins. LFA-1 thus represents both a previously undescribed lymphocyte surface antigen and molecular site for blockade of CTL-mediated killing.**

Cytolytic effector T lymphocytes play crucial roles in immune resistance to intracellular parasites, viruses, and neoplasms, yet the biochemical nature of their antigen receptor and the nature of their cytolytic attack mechanism remain obscure. Physiologic and pharmacologic studies of the interaction between immune cytolytic T lymphocytes (CTL) and specific antigen-bearing target cells have shown that CTL-mediated killing is a complicated multistep process (1–5). Classical biochemical methods of correlating these functions with specific structures would involve the purification and measurement of activity of isolated components. These methods have not been appropriate to the CTL, because disrupted CTL or CTL supernatants have been unable to mediate lytic activity (6–8).

An attractive alternative approach is the use of monospecific antibodies as *in situ* probes for CTL surface molecules that participate in the killing pathway and for the isolation and chemical characterization of these molecules. Recently, Shinohara *et al.* (9) demonstrated that, in the absence of complement, antisera to Lyt-2 significantly inhibit CTL-mediated killing. This finding has been confirmed by a number of investigators with classical and monoclonal antibodies to the Lyt-2,3 complex (10–14).

The advent of technology for producing hybridomas secreting xenogeneic monoclonal antibodies (MAb) (reviewed in ref. 15) provides the opportunity to define further molecules involved in CTL function with the use of CTL-blocking MAb reagents. Previously, xenoimmunization of rats with mouse spleen cells followed by cell hybridization and cloning has been used to obtain MAb defining a number of antigens expressed on T lymphocytes. These include the common leukocyte antigen (16, 17), also known as T200 (18) or Ly 5 (19, 20), Lyt-1, Lyt-2, Thy-1 and Lgp100 (21), and H-2 (17). In this paper, we have extended this approach to obtain a panel of 24 anti-CTL MAb defining at least 14 different antigens expressed on these cells. The MAb have been tested in the absence of complement for inhibition of cell-mediated killing.

We report that MAb to 12 different surface components have little or no effect on CTL-mediated killing, and confirm inhibition by MAb to Lyt-2,3. Furthermore, we report the identification and structure of a previously undescribed CTL surface antigen that is suggested by inhibition experiments to participate in CTL-mediated killing.

MATERIALS AND METHODS

Media and Reagents. The assay medium (L15HGS) was L15 medium (Microbiological Associates, Bethesda, MD) containing 5% heat-inactivated fetal calf serum, D-glucose (2 mg/ml), 10 mM Hepes buffer (GIBCO), penicillin at 100 units/ml, and streptomycin at 100 μg/ml.

For *in vitro* generation of effector cells, the culture medium was RPMI 1640 with the following additions: 2 mM extra L-glutamine, 2 g of extra D-glucose per liter, penicillin at 100 units/ml, streptomycin at 100 μg/ml, 50 μM 2-mercaptoethanol (Eastman Kodak), and 2% heat-inactivated fetal calf serum.

Cells. Brown Norway (BN) rat lymphoma (BNLΦ) target cells were maintained in culture in RPMI 1640 medium with 5% heat-inactivated fetal calf serum and labeled with ^{51}Cr as described (22).

C57BL/6 anti-BNLΦ (xenogeneic) effector cells were gen-

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Abbreviations: B6, C57BL/6J mouse strain; BN, brown Norway rat strain; BNLΦ, BN rat lymphoma; Con A, concanavalin A; CTL, cytolytic T lymphocyte(s); LFA, lymphocyte function-associated antigen; MAb, monoclonal antibody (antibodies).

* Present address: Sidney Farber Cancer Institute, Harvard Medical School, Boston, MA 02115.
† Present address: Dept. of Microbiology, Univ. of Massachusetts, Amherst, MA 01103.
‡ To whom reprint requests should be addressed.

erated by *in vitro* secondary stimulation in culture. Spleens from C57BL/6 (B6) mice immunized intraperitoneally with 10^7 BNLΦ viable cells 1–3 months previously were harvested aseptically and minced, and the splenocytes were washed with medium three times for use as responders. Stimulators were BNLΦ cells that were irradiated with 1600 roentgens (1 roentgen = 2.6×10^{-4} coulomb/kg) from a Gammacell-40 cesium source Research Irradiator (Atomic Energy of Canada Limited, Ottawa, ON, Canada), and subsequently washed three times. Responders (8×10^7) were cultured with 2×10^6 stimulators in plastic tissue culture flasks (no. 25100, Corning) in a total volume of 25 ml. Flasks were incubated upright at 37°C in a humidified atmosphere of 5% CO_2 in air for 4–6 days.

Cells from *in vitro* cultures were harvested, washed, and resuspended in 2 ml of assay medium. Nonviable cells were removed by layering the harvested cells over 3 ml of lymphocyte separation medium (Bionetics, Kensington, MD) and centrifuging at 500 × *g* for 7 min at room temperature. Cells were collected from the interface, washed three times, and resuspended in medium. Viable cell numbers were determined by trypan blue exclusion.

Immunization and Fusion. Two (Lewis × BN)F_1 14-month-old male rats were primed intravenously with 1.5×10^7 B6 anti-BNLΦ lymphocytes (prepared as described above, and including CTL) in 1 ml of medium plus 1% normal rat serum on day −73. The B6 CTL used for immunization produced 50% corrected ^{51}Cr release at a lymphocyte-to-target-cell ratio of 13 in 2 hr ($R^{2\,hr}_{50\%} = 13$). On day −9, one rat (for fusion M5) was boosted intravenously with 0.9×10^7 B6 anti-BNLΦ lymphocytes ($R^{2\,hr}_{50\%} = 1.3$) and on day −3 both rats were boosted intravenously with 1.8×10^7 B6 anti-BNLΦ lymphocytes ($R^{2\,hr}_{50\%} = 9$). On day 0, NSI myeloma cells were fused with spleen cells from the tertiary immunized rat (fusion M5, spleen cell-to-myeloma cell ratio = 5.4) and from the secondary immunized rat (fusion M7, spleen-to-myeloma ratio = 10) with 50% (wt/wt) polyethylene glycol as described (17, 23). Cells were distributed into 1620 0.2-ml wells in 96-well plates (Costar) and fed with 20% fetal calf serum/Dulbecco's modified Eagle's medium/hypoxanthine/aminopterin/thymidine as described (17).

Inhibition of Cell-Mediated Cytolysis by MAb. Effector cells (50 μl) were pretreated with 50 μl of monoclonal hybridoma culture supernatant or medium in 12 × 75 mm plastic tubes (no. 2052, Falcon) for 30 min at room temperature, vortex mixing at 0 and 15 min. (The culture supernatant had been dialyzed against L15HG medium at 4°C for 24 hr with two changes of medium.) On ice, 1×10^4 of the appropriate ^{51}Cr-labeled target cells were added in 50 μl. All tubes were Vortex mixed, centrifuged at 4°C for 5 min at 500 × *g*, and then incubated at 37°C for 2–4 hr. Corrected percentage ^{51}Cr release was determined and calculated as $100(e - c)/(100 - c)$ as described (24) (*e* and *c* are the percentage ^{51}Cr release in the presence and absence of effector cells, respectively).

Other Methods. The ^{125}I-labeled anti-rat IgG indirect binding assay, immunoprecipitation, cloning and growth of hybrid cells, and fluorescent labeling and fluorescence-activated cell sorting analysis were carried out as described (16, 25).

RESULTS

Strategy for Eliciting and Assaying Anti-CTL MAb. To prime for hybridoma production (Lewis × BN)F_1 rats were immunized with mouse anti-rat (B6 anti-BNLΦ) CTL-rich living cell populations. Spleen cells from immune rats were fused with NSI mouse tumor cells, distributed among 1620 culture wells, and grown as previously described (17). Culture supernatants were screened for MAb binding preferentially to activated T lymphocytes compared to B lymphocytes by using an indirect binding assay (Fig. 1). Cloning was attempted from all cultures with T/B specificity ratios of 4 or higher, and 14 stable cloned lines were successfully isolated (Fig. 2*A*). Some of the cultures with specificity ratios less than 4 were also selected for cloning. On the basis of immunoprecipitation, clones recognizing Ia antigens and an antigen on B and T lymphocytes apparently identical to Lgp100 (21) were obtained (Fig. 2*B*). One of several highly lytic clones was selected for further testing (Fig. 2*C*).

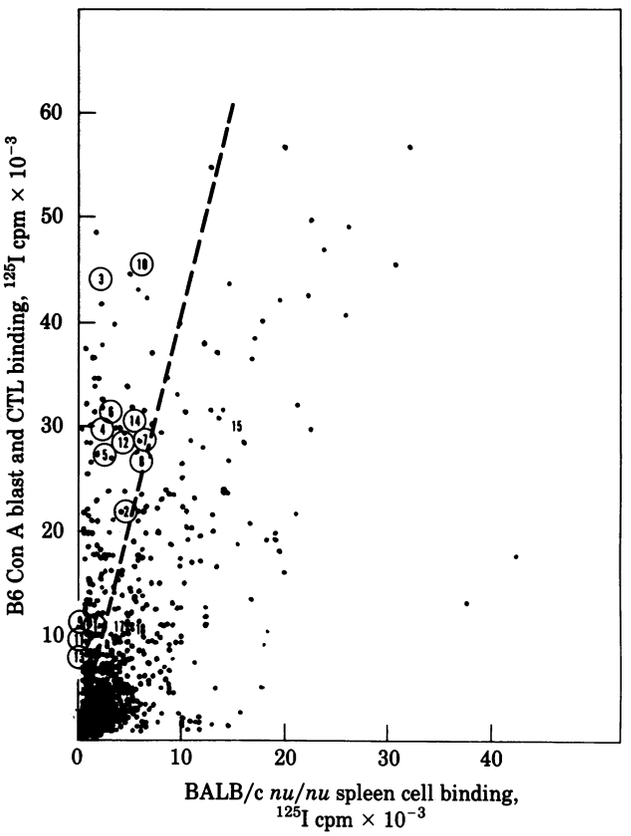

FIG. 1. T cell versus B cell binding activity of M5 and M7 culture supernatants in the indirect ^{125}I-labeled anti-Ig assay. M5 and M7 culture supernatants (10 μl) were assayed on 5 μl of a mixture of 9.25 × 10^7 concanavalin A (Con A)-stimulated blasts per ml and 0.5×10^7 B6 anti-BNLΦ CTL per ml (T cells) (ordinate) or on 10×10^7 BALB/c *nu/nu* spleen cells per ml (B cells) (abscissa). Positive control supernatants binding to both B and T cells (M1/9.3 and M1/42, see Fig. 2) gave T/B ratios of 1.7 and 2.0, respectively. Therefore, cultures with ratios >4 (to the left of the broken line in the figure) showed T cell preference and were selected for cloning. Lines that have been used in this paper (all cloned and subcloned) are marked by numbers, and are circled if the original criterion for cloning was by this binding assay, or uncircled if selection was for immunoprecipitation or complement-dependent lysis (see Fig. 2): 1, M5/24; 2, M5/35; 3, M5/49; 4, M5/54; 5, M5/56; 6, M5/69; 7, M5/78; 8, M5/106; 9, M7/5; 10, M7/7; 11, M7/8; 12, M7/14; 13, M7/20; 14, M7/21; 15, M5/114; 16, M7/81; 17, M7/83; 18, M7/86.

In testing the effect of MAb on CTL-mediated killing, it was important to utilize a system in which MAb did not bind to the target cell. Antibodies bound to target cell antigens have previously been demonstrated to inhibit CTL-mediated lysis (reviewed in ref. 1). Therefore, a xenogeneic mouse CTL anti-rat tumor target cell system was utilized (MAb and target cells of the same species).

Killing of rat BNLΦ target cells by mouse B6 lymphocytes in this system is mediated by CTL, because (*i*) the effector cells are Thy-1^+ and Lyt-2^+ (Table 1), and (*ii*) killing is specific for

Table 1. B6 anti-BNLΦ killers are Thy-1$^+$ Lyt-2$^+$ CTL

Effector cell treatment	% corrected ^{51}Cr release
None	19.9 ± 0.5
C	18.5 ± 0.7
Anti-Lyt-1 + C	20.2 ± 0.5
Anti-Lyt-2 + C	2.6 ± 0.2
Anti-Thy-1 + C	1.7 ± 0.3

Killer cells were incubated with equal volumes of anti-Lyt-1 or anti-Lyt-2 MAb supernatants (21) or with anti-Thy-1 MAb ascites (26) diluted 1:1000 for 30 min at 22°C, then with guinea pig complement (C) for 30 min at 37°C, and then assayed for lysis of ^{51}Cr-labeled BNLΦ cells. Release is presented as mean ± SEM.

the sensitizing BN rat cells: (Lewis × BN)F_1, but not Lewis target cells are killed (unpublished data).

To test for inhibition of CTL-mediated killing, dialyzed MAb were preincubated with effector cells in the absence of complement, ^{51}Cr-labeled target cells were added, and specific ^{51}Cr release was measured after 2–4 hr at 37°C. MAb obtained here and previously in this (16, 17) and other laboratories (21) were tested (Fig. 2). Of 24 MAb, 21 had little or no effect on killing (−8% to +26% inhibition). These included MAb to Thy-1, Lyt-1, Ly 5 [= T200 (19, 20); = common leukocyte antigen (27)], Ly 6, H-2, a 95,000 M_r polypeptide apparently identical to Lgp100 (21), previously undescribed antigens with polypeptide chains of M_r 140,000 and 250,000, 115,000, 60,000, and 46,000, and two other antigens defined by M5/69 and M7/86 with distinctive cell distributions but not yet structurally characterized.

Antibodies to the Lyt-2,3 antigen complex have previously been reported to block allogeneic CTL-mediated lysis (9–14), and we confirmed this in the xenogeneic anti-BNLΦ system. The 53.6 (21) and M5/24 anti-Lyt-2,3 MAb gave 90% and 40% inhibition of killing, respectively (Fig. 2).

The final MAb, M7/14, gave highly reproducible 90% inhibition of CTL-mediated killing (Fig. 2). Even when high CTL-to-target-cell ratios or highly active CTL preparations were employed, which gave 79–93% ^{51}Cr release, 94% inhibition was observed in the presence of M7/14 (Table 2). Because these results suggest an important role in cell-mediated killing, the antigen defined by M7/14 MAb has been designated a *lymphocyte function-associated antigen*, or LFA-1.

The xenogeneic CTL assay system employed here was de-

Table 2. Inhibition of B6 anti-BNLΦ T lymphocyte-mediated cytolysis by M7/14 supernatant

Number of observations	Corrected % ^{51}Cr released without M7/14		% inhibition by M7/14	
	Range	Mean	Range	Mean
8	13–20	16.1	32–97	73.0
19	23–45	32.8	64–104	89.1
12	46–68	61.4	51–104	88.0
6	79–93	85.0	88–99	94.0

Experiments utilized effector-to-target-cell ratios ranging between 2:1 and 60:1. Each observation represents the mean of a duplicate or triplicate test. Data were taken from 12 separate experiments performed on different days. Data from 4 experiments were excluded due to spontaneous ^{51}Cr release exceeding 25%, and data from 1 experiment were excluded due to variability in spontaneous release. For all observations included, spontaneous release of ^{51}Cr in tubes containing M7/14 but with effector cells omitted was 10–24%. Data for M7/14 supernatants and purified IgG are pooled, because no differences were evident.

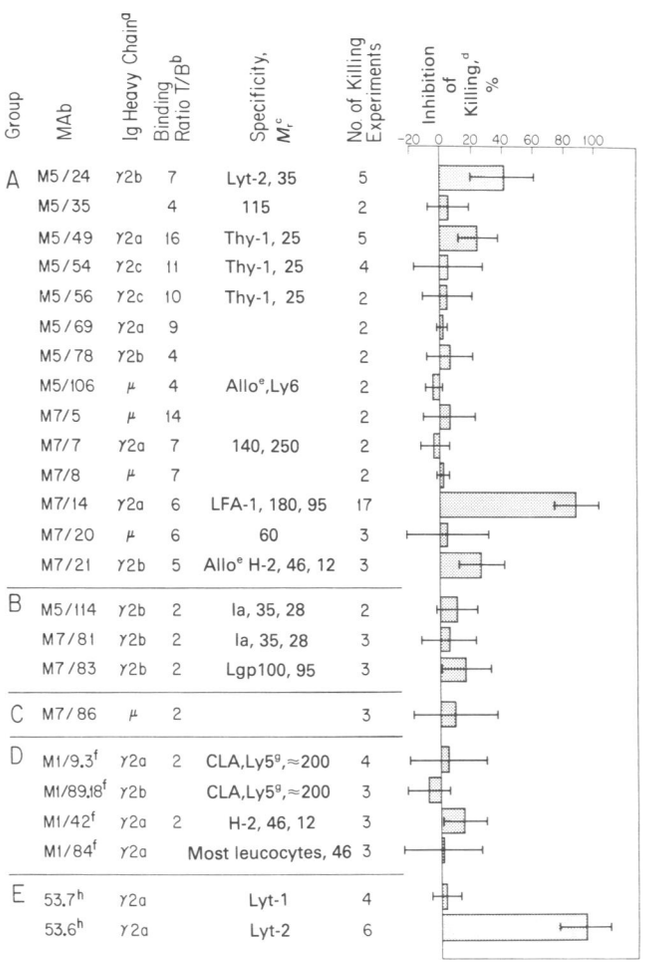

FIG. 2. Effects of monoclonal antibodies on B6 anti-BNLΦ CTL-mediated killing. Effector cells were pretreated with an equal volume of dialyzed hybridoma culture supernatant for 30 min at 20°C, then ^{51}Cr-labeled BNLΦ cells were added and the assay was completed. Data were included from all experiments and all sensitized-to-target cell ratios in which specific ^{51}Cr release in the absence of antibody was in the range 20–70% and in which spontaneous ^{51}Cr release in the presence of antibody but the absence of effector cells was ≤25%. One or two means of duplicate or triplicate observations were included from each experiment. Percent inhibition of corrected ^{51}Cr release is expressed relative to cultures treated with dialyzed NSI culture supernatant; this supernatant produced a variable augmentation of corrected ^{51}Cr release (relative to medium controls) with a mean ± SD of 18 ± 20%. MAb are grouped according to criteria used for isolation or their source: (*A*) Selected for ratio >4 of indirect binding to B6 Con A-stimulated blasts vs. *nu/nu* BALB/c spleen; two clones later proved to be allospecific rather than T cell specific. (*B*) Immunoprecipitation of ^{125}I-labeled polypeptides differing from those recognized by the previously obtained MAb listed in *D*. (*C*) Lysis of P815 and EL-4 tumors. (*D*) Previously characterized MAb (17) reactive with both B and T cells. (*E*) Lyt-1 and Lyt-2 MAb described by Ledbetter and Herzenberg (21).

[a] Determined by double immunodiffusion as described (17).

[b] Ratio of ^{125}I-labeled rabbit anti-rat IgG bound in the indirect binding assay to MAb-coated (B6 Con A-stimulated splenoblasts)/(*nu/nu* BALB/c splenocytes); see Fig. 1.

[c] Molecular weight was estimated by immunoprecipitation of lactoperoxidase ^{125}I-labeled B6 Con A-stimulated splenoblasts followed by reduction and $NaDodSO_4$/polyacrylamide gel electrophoresis. $M_r \times 10^{-3}$ is tabulated.

[d] Inhibition of specific ^{51}Cr release from BNLΦ target cells. Error bars indicate ±SD.

[e] Found to recognize an allospecificity present on B6 and absent on BALB/c mouse splenocytes.

[f] Ref. 17.

[g] Refs. 19 and 20.

[h] Ref. 21.

Table 3. BNLΦ cells do not express M7/14 antigen[a]

Cells	M7/14⁺, %	Average fluorescence intensity[b] M7/14	Control
BNLΦ	0	0.400	0.404
Mouse CTL preparation (secondary B6 anti-P815)	96	7.5	0.3

[a] Cells were labeled with M7/14 MAb supernatant or NSI culture supernatant and rat IgG at 50 μg/ml (control), then with fluorescein-conjugated anti-rat IgG, and analyzed on the fluorescence-activated cell sorter.

[b] Integrated average, relative to intensity of glutaraldehyde-fixed sheep erythrocytes.

signed to exclude the possibility that MAb could bind to target cells and thereby exert an inhibitory effect. However, it was important to test the possibility that the M7/14 rat MAb might be autoreactive with the rat BNLΦ target cells. Indirect immunofluorescence analysis (Table 3) demonstrated no binding of M7/14 to BNLΦ cells, although binding of as little as 3% of the amount seen with CTL effectors would have been readily detectable.

To determine the structure of the LFA-1 antigen defined by M7/14, lysates of ^{125}I-surface-labeled B6 anti-BNLΦ CTL cell preparations were immunoprecipitated with M7/14 IgG and analyzed by NaDodSO$_4$/polyacrylamide gel electrophoresis and autoradiography (Fig. 3*A*). Two specifically precipitated polypeptide chains were obtained, an α chain of 180,000 M_r and a β chain of 95,000 M_r. The same α and β chains were also precipitated by M7/14 from Con A-stimulated blasts (Fig. 3*B*, lane 3). Furthermore, LFA-1 is clearly distinct from Ly-5, which has chains of 200,000 and 180,000 M_r (Fig. 3*B*, lane 4), and from Lyt-2,3, which has chains of 35,000–30,000 M_r (Fig. 3*B*, lane 7).

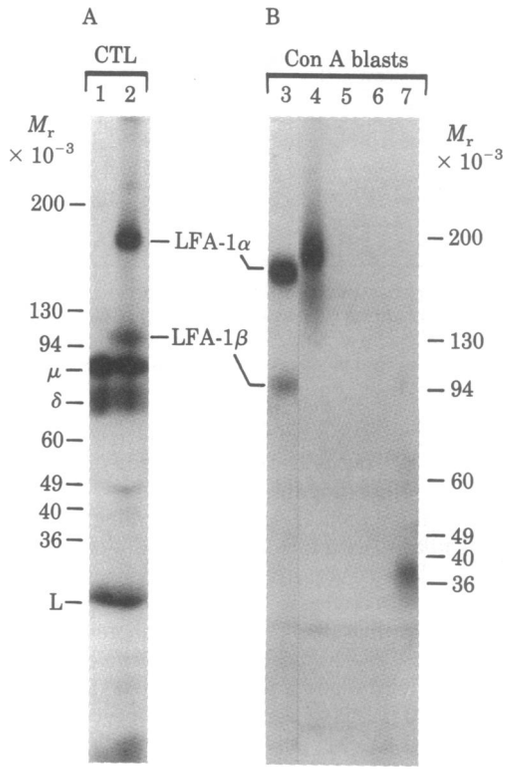

FIG. 3. NaDodSO$_4$/polyacrylamide gel electrophoresis of LFA-1, Ly-5, and Lyt-2,3 immunoprecipitated by MAb. Spleen cells from B6 mice primed with BNLΦ cells and restimulated *in vitro* (CTL, *A*), or B6 spleen cells stimulated with Con A at 2 μg/ml for 3 days (Con A blasts, *B*) were surface labeled with ^{125}I by using Iodogen (Pierce) (28). Cell lysates were immunoprecipitated with NSI supernatant plus added normal rat IgG as control (lanes 1 and 6), 20 μg of M7/14 IgG (lanes 2 and 3), or culture supernatants containing the M1/9.3 anti-Ly 5 (or common leukocyte antigen) MAb (lane 4), the nonprecipitating M7/87 MAb (lane 5), or the anti-Lyt-2,3 M5/24 MAb (lane 7), and addition of rabbit anti-rat IgG. Reduced samples were subjected to NaDodSO$_4$ electrophoresis in a 5–15% polyacrylamide gradient gel and autoradiography. In addition to specifically precipitated LFA-1 α and β chains, mouse μ, δ, and light (L) chains from B cells in the CTL preparation (*A*) were precipitated by crossreaction with rabbit anti-rat IgG. Mouse surface Ig was absent in *B* because the lysate was precleared with rabbit anti-rat IgG and anti-mouse IgG.

DISCUSSION

In this report, MAb have been used to probe for functionally important molecules on the CTL cell surface. The central idea in these experiments was that MAb binding to a structure involved in CTL-mediated lysis should cause either steric hindrance or removal of the structure from the surface through modulation, thereby inhibiting killing. On the other hand, binding to a CTL surface structure that did not participate in the killing pathway should have no effect. It was important to use a CTL assay system in which the MAb did not bind to target cells, because MAb to target antigens such as H-2 are well known to block CTL-mediated killing (1, 29, 30). Therefore, we tested the rat anti-mouse MAb for inhibition of mouse CTL-mediated lysis of rat target cells. In this doubly xenogeneic assay system, the MAb and target cells are both of rat origin and therefore binding of MAb to the target cell should not occur.

Antigens on the CTL surface may be placed into two groups on the basis of the effect on CTL-mediated killing of MAb bound to them. Three MAb to Thy-1, two to H-2, and single MAb to Lgp100, Lyt 1, Ly 6, previously undefined polypeptide chains of M_r 115,000, 140,000 and 250,000, 60,000, and 46,000, and at least two other distinct antigens (M5/69 and M7/86 antigens) had little or no effect on CTL-mediated killing. Many antibodies to known or possible CTL membrane antigens have previously been shown not to block killing. These include conventional anti-H-2 and anti-Ig antisera (ref. 10 and reviewed in ref. 1), monoclonal anti-H-2 antibodies (29, 30), anti-β_2-microglobulin (31, 32), anti-Thy-1 (10, 11, 32), anti-Ia (32), anti-TL (10), antisera to various murine leukemia virus glycoproteins (10), two independent xenoantisera raised against mouse CTL (refs. 33 and 34, as elaborated on p. 216 in ref. 32), anti-Lyt-1 (10) as well as numerous other alloantisera (9), and even anti-idiotypic antisera with demonstrated anti-receptor activity for other T cell functions (refs. 35–38, p. 140 in ref. 39). Two MAb to a common leukocyte antigen, identical to T200 (18) and Ly 5 (19, 20), were also found here to have no effect on killing, in contrast to a previous report employing alloantiserum to Ly-5 (Lyt-4) (10). This discrepancy could be due to recognition of different portions of the same molecule, or to additional antibodies present in the noncongenic Ly-5 antiserum (10) specific for other CTL or target cell determinants.

Lyt-2,3 antisera and MAb had previously been shown to inhibit allogeneic CTL-mediated killing, (10–13), and in some of these studies binding to target cells was genetically excluded. The present studies confirmed, as expected, that MAb to Lyt-2 also inhibit xenogeneic CTL-mediated killing.

The most interesting result of this report concerns the M7/14 MAb. This MAb gave highly reproducible ≈90% blockade of CTL-mediated killing. Inhibition is mediated by binding to the CTL effector rather than the target cell, as expected from the doubly xenogeneic MAb and CTL assay systems employed,

and as explicitly demonstrated by quantitative immunofluorescence flow cytometry. M7/14 defines a cell surface antigen, termed LFA-1, with two polypeptide chains of 180,000 and 95,000 M_r. Neither the antigen nor its association with CTL-mediated killing has been described previously. This antigen is distinct from glycoproteins of 145,000 M_r (40) and 11,000 M_r (41) previously identified on T lymphocyte subpopulations with CTL activity (40). Direct comparisons in $NaDodSO_4$/polyacrylamide gel electrophoresis have confirmed that LFA-1 is distinct from T200 = (common leukocyte antigen = Ly 5) and from Lyt-2,3. M7/14 MAb also strongly inhibits allogeneic and anti-modified self-CTL-mediated killing (42).

Inhibition by anti-LFA-1 and anti-Lyt-2 MAb of CTL-mediated killing is not due to nonspecific blanketing of the CTL surface by MAb, because anti-H-2 and anti-Thy-1 MAb of the same subclass have little effect but bind in 2.5–10 times higher amounts (27). Nor does M7/14 MAb agglutinate CTL (42). This highly specific inhibition suggests LFA-1 and Lyt-2 either directly participate in or are closely associated with the mechanism of cell-mediated killing.

CTL-mediated killing is a multistep process (1–3, 43), which involves crawling or surface motility during target engagement (4, 44–46), specific antigen recognition (5), adhesion (4, 22), lethal-hit triggering (47, 48), and delivery of the lethal hit (4, 49). It is likely that a number of surface proteins are involved in this cell-mediated killing pathway and that antibodies bound to any one of them would block killing. Some components of the cell-mediated killing pathway may be shared with other functional pathways and thus be expressed also on other types of cells. It should be emphasized that the data presented here suggest participation of LFA-1 in CTL-mediated killing but do not bear on whether LFA-1 expression is limited to CTL. In fact, elsewhere we will demonstrate that LFA-1 is also expressed on non-T lymphocytes (27). The latter finding suggests that LFA-1 does not mediate antigen recognition but participates in some other step in the killing functional pathway. The M7/14 MAb should be useful for better defining this step and for the isolation and detailed biochemical characterization of LFA-1. Further studies with CTL-blocking MAb probes should provide a means for understanding the biochemical basis of CTL antigen recognition and the cytolytic attack mechanism.

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